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Touch DNA – the prospect of DNA profiles from cables

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Title Page

Touch DNA – the prospect of DNA profiles from cables

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Touch DNA – the prospect of DNA profiles from cables

Metal theft in the railroad industry poses significant challenges to transport investigators. Cable sheaths left behind at crime scenes, if appropriately analyzed, could provide valuable evidence in a forensic investigation, but attempts at recovering DNA are not routinely made. Experiments were set up to ascertain the success in DNA recovery from the surface of cable sheaths after deposition of a) sweat; b) extracted DNA and c) fingermarks. Since investigators try to collect fingermarks and often treat the cables with cyanoacrylate fuming (CNA fuming) or wet powder suspensions (WPS) to enhance the marks this study investigated the recovery of DNA from fingermarks pre and post enhancement. The double swab technique and mini-taping were compared as options to recover DNA from the cable sheaths. Results demonstrate that generally, there is no significant difference between using swabs or mini-tapes to recover the DNA from the non-porous cables ($p > 0.05$). It was also illustrated that CNA fuming performed better than WPS in terms of subsequent recovery and profiling of DNA. CNA fuming resulted in an average increase in DNA recovered via swabbing and taping (more than 4x and 8x respectively), as compared to no treatment, with 50% of the DNA recovered after CNA fuming generating full DNA profiles.

Keywords: Touch DNA; fingermarks; cyanoacrylate fuming; wet powder suspensions; DNA profiling.

1. Introduction

Metal theft (the stealing of items for its constituent metal parts) is becoming more prevalent, due to an increase in the value of scrap metal. Main targets of metal theft are electrical equipment, construction sites and public infrastructure. The railroad industry is the biggest victim of metal theft, with more than double the incidents occurring at railways than in the communications trade. The industry estimates annual losses of tens of millions of British pound sterling from cable theft (British Transport Police, personal communications, 2015). Cable thefts are also a key disruption in rail services, not only are commuters greatly inconvenienced with train delays, but they are also potentially in danger if the theft causes the train to malfunction. The perpetrators themselves may be putting themselves at high risk of electrocution and even death.

In most cases, the cable sheaths are removed from the cable and discarded at the theft site. Since these have been handled by the perpetrators, they have the potential to provide trace evidence (in the form of touch DNA) that can then help investigators link suspects to the scene (British Transport Police, personal communications, 2015). Touch DNA is DNA transferred from a person to an object via contact with the object [1-3] which, most notably in the case of metal/cable theft, comes from sweaty hands. The DNA from touch DNA arises from nucleated skin cells (keratinocytes) through incomplete degradation during the keratinocyte differentiation process, epithelial cells through contact of the hands with other body parts and cell free DNA [4-6]. There are many variables that affect the amount of touch DNA recovered from an exhibit, such as the propensity of individuals in leaving behind their DNA, the surface of interest and the time the DNA is exposed to external factors [5, 7-10].

As both fingermarks and DNA can be used as evidence for identification purposes, relevant to this subject is also the ability to obtain a useful DNA profile after reagents to enhance fingermarks have been applied. In general, there are three major steps in dealing with fingermarks/DNA evidence analysis. First, there is a practical need for localisation/enhancement of the fingermark/DNA followed by recovery via a suitable technique, and finally analysis of the fingermark/DNA. The surfaces commonly encountered in cable theft are made of black plastic polymers. Latent fingermarks on this type of material are visualised through cyanoacrylate fuming (CNA fuming) or wet powder suspensions (WPS) as it has been shown that both techniques perform equally well [11]. Studies have been reported on the recovery of DNA from fingermarks deposited on different surfaces. The results often showed that the possibility to obtain genetic profiles from touched items strictly depends on the surface type [12] in addition to the enhancement technique employed [13]. Plastic materials have often been employed in such studies with donor profiling information being successfully recovered [12, 14-15]. However, no

studies have been conducted to investigate the deposition, recovery and profiling of touch DNA from cable sheaths after treatment with CNA and WPS. As cyanoacrylate and wet powders are not chemically destructive to the DNA [16-17], it may be more practical for investigators to first carry out the enhancement and recovery of latent fingerprints before pursuing the prospect of DNA evidence. Even if the development process fails to reveal any usable fingerprints, it will reveal areas on the cable where it has been grasped and therefore a potential site of DNA deposition. The present study compares the efficiency of the double swab technique against that of mini-taping in the recovery of touch DNA from cables, and the possibility of obtaining DNA profiles from touch DNA after treatment with CNA fuming or wet powder suspensions. Results from this work can contribute to enabling targeted recovery and profiling of touch DNA.

2. Materials and Methods

DNA was applied in one of three forms: deposition of sweat from a stock solution, deposition of extracted DNA from a stock solution or deposition of touch DNA (fingerprints) from donors. All the DNA sources used in the present work (sweat, extracted DNA and touch DNA) were from individuals other than those that prepared the cables, collected and processed the samples. Positive controls (i.e. buccal swabs) were collected from each of the donors involved in the study so that comparisons could be made and the origin of the DNA profiles confirmed.

2.1 Cable preparation and analysis

A 7 m long, smooth non-porous black cable (approximately 0.02 m diameter), was supplied by the British Transport Police. This cable was cut into shorter pieces varying in lengths of 0.36-0.4 m. Boxes of dimensions 1.5 cm × 3 cm were drawn on the cable using a permanent marker to highlight the areas for DNA deposition. Before DNA deposition, the cables were thoroughly cleaned with DNA-ExitusPlus™ IF (PanReac AppliChem, Germany). Double gloves were also worn in the handling of the cables in order to limit contamination risk. Negative control samples were taken and although some of them showed trace amounts of DNA at the quantification step, no profile was obtained during DNA analysis.

2.1.1 Collection of sweat

Sweat was collected in six 1.5 mL microcentrifuge tubes over a couple of days after a gym workout, from the volunteer's forehead and arms. The collected sweat was pooled into a larger 15 mL centrifuge tube and used as a sweat stock solution. In order to imitate real cases, sweat was used directly without prior extraction. From our preliminary studies, an average of 63.1 ng of DNA was extracted from 200 µL of sweat (data not shown). Twenty repeats of 32 µL of sweat (approximately containing 10 ng of DNA) were deposited on the cables for comparison between swabbing and taping recovery methods.

2.1.2 Extracted DNA

DNA was extracted from sweat and buccal swabs. The extracted DNA was pooled together then quantified to create a stock solution of 23.2 ± 2.3 ng/µL (data not shown). The extracted DNA was diluted to reproduce deposition of approximately 10 ng of extracted DNA in a 32 µL aliquot, on the cables. Similarly, twenty repeats were performed, 10 each for swabbing and taping.

2.1.3 Collection of touch DNA

In order to mimic real scenarios, donors were requested to deposit their fingerprints on the cables without prior washing of their hands. They were only asked to rub both hands together to reduce intra-variability. Five fingers from both hands were used, in a rolling motion, back and forth, to deposit the touch DNA. The collection was done one finger at a time. Rather than the whole finger, only the top sections of the fingers and thumbs were deposited. In this paper, a 'fingerprint' is referred to as the mark left behind by the top one third of a finger (or top half of a thumb). A total of 120 fingerprints from six donors were collected on different days to assess 'pre-treatment recovery of DNA' and 'post-

treatment recovery of DNA'. All fingerprints deposited were recovered on the same day of deposition and both lifts and swabs stored in the freezer in extraction tubes.

2.2 Enhancement of marks

A proportion of the fingerprints (60 marks) deposited were chemically treated with either CNA fuming or WPS to enable the latent marks to be visible, so as to achieve targeted recovery of DNA. CNA fuming was carried out in an MVC 5000 (Foster & Freeman, U.K.) superglue fuming cabinet with relative humidity between 75-90%. VC363 Cyanoacrylate (Tetra Scene of Crime, U.K.) was heated to 120°C and the fumes were allowed to circulate for 13 minutes before being purged. Due to the black surface of the cable, White Wet Powder™ (Kjell Carlsson Innovation, Sweden), a titanium dioxide based white powder suspension was selected to provide the contrast needed for the marks to be visible. The cables were pre-rinsed with running tap water and the suspension applied with a squirrel-hair brush. The surface was exposed to the suspension for approximately 10 seconds before the excess was removed with running tap water. The cables were allowed to dry in a fume cupboard for 30 mins before any DNA recovery work was carried out.

2.3 Recovery of DNA

Recovery of DNA from the cables was carried out with either one of two techniques: double swab technique [18] or mini-taping [19]. With the double swab technique, a wet cotton swab was used to swab the area of interest, followed by a second dry cotton swab. Both swabs were cut off and placed in the same extraction tube for downstream processes. The amount of fluid on the wet cotton swabs was standardised by pipetting 20 µL of sterile distilled water on the swab prior to swabbing. All swabs used were 6" Forensic Woodstick Cotton Sharpened Tip sterile swabs (Technical Service Consultants, U.K.). For mini-taping, one mini-tape (WA Products, U.K.) was applied to the area of interest with medium pressure a total of 4 times to ensure the whole area was covered. Each tape was then placed in an extraction tube in a rolled fashion, with the adhesive side facing inwards to avoid sticking to the walls of the tube. Caution was also taken to ensure that the adhesive side of the tapes were not self-sticking in the tubes.

2.4 DNA extraction and quantification

DNA recovered with swabs and mini-tapes was extracted with QIAamp® DNA Investigator Kit (Qiagen U.K.) in accordance with the manufacturer's recommendations; the final elution volume was reduced from 50 µL to 20 µL. Quantitation of DNA was carried out in the ABI Prism 7000 real-time PCR instrument using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems™, U.K.), in a 14 µL reaction volume which includes 2 µL of DNA.

2.5 DNA amplification and analysis

Samples showing sufficient amounts of DNA at the quantification stage were profiled. The DNA profiling was conducted with the PowerPlex® ESI 17 Pro System (Promega, U.K.) kit in 7 µL reaction volumes, consisting of 1 µL of template DNA (0.5 ng/µL). In cases where the DNA concentration was below the optimum concentration (< 0.5 ng/µL), the amount of template DNA was increased to 5 µL, with template DNA replacing the water component in the reaction volume. Amplification was carried out as per the manufacturer's recommendations at 28 cycles, in a 9700 thermal cycler (Applied Biosystems™, U.K.). The amplified products were then run in an ABI Prism 3130 Genetic Analyzer and the profiles analysed with the GeneMapper Software (Applied Biosystems™, U.K.). The peak height threshold was 50 relative fluorescent units (RFU).

3. Results

3.1 DNA Quantitation

3.1.1 Preliminary studies on sweat and extracted DNA

A preliminary study was conducted to determine if it was possible to recover DNA from the cable type used in the study. Whole sweat and extracted DNA were used in order to place a known amount of DNA on the cable before attempting to recover it. The efficacy of the double swab technique and mini-taping in the recovery of sweat and extracted DNA was compared by using a two-tailed independent t-test.

Table 1

Summary of average amounts of DNA recovered from sweat and extracted DNA using either the double swab technique or the mini-taping technique (n=10) and corresponding p-values from statistical tests. ExDNA = extracted DNA.

Method	Sweat Swab	Sweat Tape	ExDNA Swab	ExDNA Tape
Average amount of DNA (ng)	7.60	8.00	4.73	6.86
Independent T-test		p-value		
Sweat-Swab vs. Sweat-Tape		0.75		
ExDNA-Swab vs. ExDNA-Tape		0.075		
Sweat-Swab vs. ExDNA-Swab		0.02		
Sweat-Tape vs. ExDNA-Tape		0.37		

The average amounts of DNA recovered for each group and the corresponding p-values obtained from the statistical tests are reported in Table 1. From the statistical analysis, there is no significant difference in using swabs or mini-tapes (p-value > 0.05) except where swabbing seems to work better at recovering sweat than extracted DNA. These results indicate that there is no difference between the two recovery techniques, therefore both were used in the subsequent experiments on touch DNA (fingermarks).

3.1.2 Recovery of pre-treatment touch DNA

The median amounts of DNA recovered via the double swab technique and mini-taping – from 60 fingermarks (n=30 for each method) deposited on the cables – were 0.37 ng and 0.41 ng respectively. This is portrayed in Figure 1. A two-tailed Mann-Whitney U Test indicated no significant difference between the two techniques of recovery (U = 410, p = 0.559).

Figure 1 about here

3.1.3 Recovery of post-treatment touch DNA

Post-treatment touch DNA refers to samples which have been exposed to either CNA fuming or WPS prior to DNA recovery. The two-tailed Mann-Whitney U Test conducted between the groups revealed that there are significant differences between using CNA fuming or WPS as chemical treatments for the visualization of potential touch DNA. Looking within the CNA fuming treatment group, there is no significant difference between swabbing and taping (p > 0.05). This concurs with expectations from the results obtained from our preliminary studies. However, within the WPS treatment group, there is a significant difference between using swabbing and taping (p << 0.05), with taping being the better recovery method. The median amounts of DNA recovered and p-values are summarised in Table 2.

Table 2

Summary of median amounts of DNA recovered after chemical treatments (n=15) and corresponding p-values from statistical tests.

Method	CNA Swab	CNA Tape	WPS Swab	WPS Tape
Median amount of DNA (ng)	3.06	4.54	0.00	1.47
Mann-Whitney U Test			p-value	
CNA-Swab vs. CNA-Tape			0.237	
WPS-Swab vs. WPS-Tape			0.006	
CNA-Swab vs. WPS-Swab			0.000	
CNA-Tape vs. WPS-Tape			0.001	

3.2 Effects of treatments on DNA recovery

The effects of the treatments on the quantity of DNA recovered were compared using a two-tailed Mann-Whitney U Test for two independent samples. The median amounts of DNA recovered for each group and the corresponding p-values obtained from the statistical tests are reported in Table 3.

Table 3

Summary of median amounts of DNA recovered before and after chemical treatments and corresponding p-values from statistical tests.

Method	Median amount of DNA (ng)
Pre-Swab (n=30)	0.37
Pre-Tape (n=30)	0.41
CNA-Swab (n=15)	3.06
CNA-Tape (n=15)	4.54
WPS-Swab (n=15)	0.00
WPS-Tape (n=15)	1.47
Mann-Whitney U Test	p-value
Pre-Swab vs. CNA-Swab	0.000
Pre-Swab vs. WPS-Swab	0.005
Pre-Tape vs. CNA-Tape	0.000
Pre-Tape vs. WPS-Tape	0.072

The amounts of DNA recovered for each group are reported in Figure 2. From the statistical analysis, there is a highly significant difference in the amount of DNA recovered post-treatment (p-value << 0.05) except in the case of mini-taping after WPS treatment.

Figure 2 about here

3.3 DNA Profiling

Table 4 summarises the DNA results obtained from the profiling of recovered fingerprints that were not exposed to any form of treatment, and those that were chemically treated by either CNA fuming or WPS. The samples were numbered randomly to provide anonymity to the donors.

Table 4

Summary of DNA profiles obtained. Profiles marked with (*) indicate mixed profiles with the number of alleles not from the donors in parentheses, (^) indicates profile without amelogenin signal, (+) indicates potential contamination issues.

Number	Method	DNA Amount (ng/μL)	Number of Alleles Detected (>50 rfu)			
			0	1-10	11-20	>20
Pre-Treatment						
1	Swab	0.023				28
2	Swab	0.009			17(1)*	
3	Swab	0.013	✓			
4	Swab	0.039			11(3)*	
5	Swab	0.150				24^
6	Swab	0.090				F
7	Swab	0.022				27
8	Swab	0.052		9		
9	Swab	0.011	✓			
10	Swab	0.113				25(3)*
11	Swab	0.010	✓			
12	Tape	0.065				21(7)*
13	Tape	0.014		7(2)*		
14	Tape	0.013	✓			
15	Tape	0.015				24(4)*
16	Tape	0.065				27(1)*
17	Tape	0.014	✓			
18	Tape	0.055				29
19	Tape	0.154				F
20	Tape	0.025				24
21	Tape	0.021	✓			
22	Tape	0.010	✓			
Post-Treatment						
1	CNA Fuming – Swab	0.053		2		
2	CNA Fuming – Swab	0.194				22
3	CNA Fuming – Swab	0.116			14	
4	CNA Fuming – Swab	0.535				F
5	CNA Fuming – Tape	0.042			12	
6	CNA Fuming – Tape	0.312				F
7	CNA Fuming – Tape	0.850				F
8	CNA Fuming – Tape	0.589				F
9	WPS – Swab	0.033		1+		
10	WPS – Swab	0.034		1		
11	WPS – Swab	0.051		4		
12	WPS – Swab	0.380				27+
13	WPS – Tape	0.165		1+		
14	WPS – Tape	0.078		7+		
15	WPS – Tape	0.036		4		
16	WPS – Tape	0.195				24+

3.3.1 Profiling of pre-treatment touch DNA

Of the 22 pre-treatment samples profiled (amount of DNA ranged from 0.009 – 0.154 ng/ μ L; 50 – 770 pg of DNA), 15 of them returned positive DNA profiles (at least one allele was detected).

The samples recovered by the double swab technique returned 5 good profiles (> 20 alleles detected) and 6 poor or null profiles.

The samples recovered by mini-taping displayed similar results, with 6 samples returning good profiles and the remaining 5 returning poor or null profiles.

3.3.2 Profiling of post-treatment touch DNA

Of the 16 post-treatment samples profiled (amount of DNA ranged from 0.033 – 0.850 ng/ μ L; 165 – 850 pg of DNA), all 16 returned positive DNA profiles. Two out of four samples recovered by swabbing after CNA fuming returned good profiles. Three out of four samples recovered by taping after CNA fuming returned full profiles. This means that sixty-three percent of the samples recovered after CNA fuming returned good, even full profiles. For the samples recovered after WPS, both swabbing and taping returned poor profiles (< 10 alleles detected). There were exceptions to this trend (number 12 and 16, Table 4 post-treatment samples). These samples returned good profiles, but were profiles of contamination (the profiles were not of the donor).

4. Discussion

4.1 Comparison of double swab technique and mini-taping

In the preliminary study using sweat and extracted DNA, it was observed that there was no difference in the DNA yield obtained from either method of recovery. The only exception was that the DNA yield from sweat was significantly higher than that from extracted DNA via the double swab technique. However, it is important to note that extracted DNA is not a good representation of the type of DNA encountered in crime scenes and real cases. Sweat would be a closer imitation of real scenarios as touch DNA comes from a mixture deposit of skin cells and sweat.

In the subsequent studies, all paired comparisons of swabbing against mini-taping within their respective groupings showed that there was no significant difference in the DNA yield. This suggests that either procedure is suitable for recovering touch DNA from cables. An exception to this was seen in the group of fingerprints treated with WPS, where mini-taping seemed to recover more DNA than swabbing.

4.2 Effects of chemical treatments

Post-treatment samples produced higher DNA yields than pre-treatment samples. Since the area of deposition and collection was kept the same for both groups, the difference is probably attributable to the post-treatment samples being visible which allowed for a more targeted collection. In pre-treatment samples, touch DNA was not visible to the naked eye and recovery of DNA was carried out based on the pre-marked areas. In real cases involving metal theft, more often than not, any touch DNA on the cable sheaths left by the perpetrators would be difficult to locate. The observation above shows the importance of the need to target specific areas of contact when trying to recover touch DNA.

Looking more specifically into the DNA yield from the two types of chemical treatments, it was observed that CNA fuming yielded substantially more DNA than WPS. This is not unexpected as the procedure of WPS involves pre-wetting the substrate which could have contributed to the mechanical removal of DNA deposits from the cable. Additionally, the brushing motion used to apply the WPS and the subsequent rinsing steps would further exacerbate this loss. Conversely, in the case of CNA fuming, there is no mechanical action involved. The treatment, which produces a white deposit, also allowed for good contrast and easy targeting of the touch DNA.

4.3 DNA profiling

There is a close relationship between the amount of DNA recovered and the ability to obtain DNA profiles. As too many variables and unknown parameters will be involved in the re-enactment of crime

scenarios, the only controlled variables in this study were the manner of contact and the type of cable substrate used. The underlying concept of this study is that if full profiles can be generated in the event of limited interaction, it is conceivable that more contact in the case of an actual metal theft would result in more DNA deposition and a higher chance of attaining full profiles [20]. Generally, across this study, samples showing higher DNA yields produced better profiles. Samples which produced null profiles came from those with DNA concentrations lower than 0.022 ng/ μ L. It is also important to note that 9 of the profiles obtained were mixed profiles. In this paper, profiles which contained mostly donor alleles are referred to as mixed profiles (denoted by the '*' notation in Table 4). This is anticipated as in the collection of touch DNA the donors were requested to leave their fingermarks without prior washing of hands. As such, DNA which does not belong to the donors may be present on their fingers and in turn transferred to the cables during deposition [3, 21], generating mixed profiles. However, this situation reflects the problem encountered in real scenarios and, in most of the mixed profiles obtained, the main contributor could be distinguished.

4.3.1 Subsequent to enhancements

Better profiles were obtained from samples after CNA fuming than those after application of WPS. Five out of eight samples returned good profiles, four of which were full profiles. In comparison, the majority of WPS treated samples returned profiles with less than 5 alleles. Samples number 12, 14 and 16 (Table 4, post-treatment samples) are excluded from this assessment as the profiles obtained were potentially profiles of contamination. Profiles with contamination issues (denoted by the '+' sign in Table 4) were profiles that were exclusive of donor alleles. Considering that these samples did not include any of the donor's alleles, and only appeared in post-treatment samples of WPS, it is likely that these alleles may have come from contaminated sources such as the brush used in WPS [22-23].

5. Conclusions

Full profiles can be obtained from the touch DNA present on the cables to assist with criminal investigations. The research has demonstrated that there is no preference in the method of recovery of touch DNA from cables – double swab technique and mini-taping are equally viable choices. With regards to the use of chemical treatments to locate areas of contact, it has been established that it is possible to obtain a full DNA profile from a single fingermark after undergoing CNA fuming treatment. CNA fuming also displays advantages of being inexpensive, easy to process and with less chance of contamination. More importantly, the enhancement allows for targeted recovery of DNA which results in larger amounts of DNA recovered and subsequently in more full profiles obtained. Wet powder suspensions on the other hand revealed disadvantages in its application procedures resulting in less DNA yields, poor profiles and contamination issues. DNA and fingerprint identification can then be applied as a two-pronged approach in criminal investigations involving cable theft.

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Captions

Figure 1: Boxplot depicting amounts of DNA recovered (ng) from untreated fingerprints, including outliers and extremes. Pre-Swab = pre-treatment recovery of DNA using double swabbing method, Pre-Tape = pre-treatment recovery of DNA using mini-taping method.

Figure 2: Boxplot depicting amounts of DNA recovered (ng) before and after chemical treatments, including outliers and extremes. Pre-Swab = pre-treatment recovery of DNA using double swabbing method, Pre-Tape = pre-treatment recovery of DNA using mini-taping method, CNA-Swab = recovery of DNA after cyanoacrylate fuming using double swabbing method, CNA-Tape = recovery of DNA after cyanoacrylate fuming using mini-taping method, WPS-Swab = recovery of DNA after wet powder suspension treatment using double swabbing method, WPS-Tape = recovery of DNA after wet powder suspension treatment using mini-taping method.

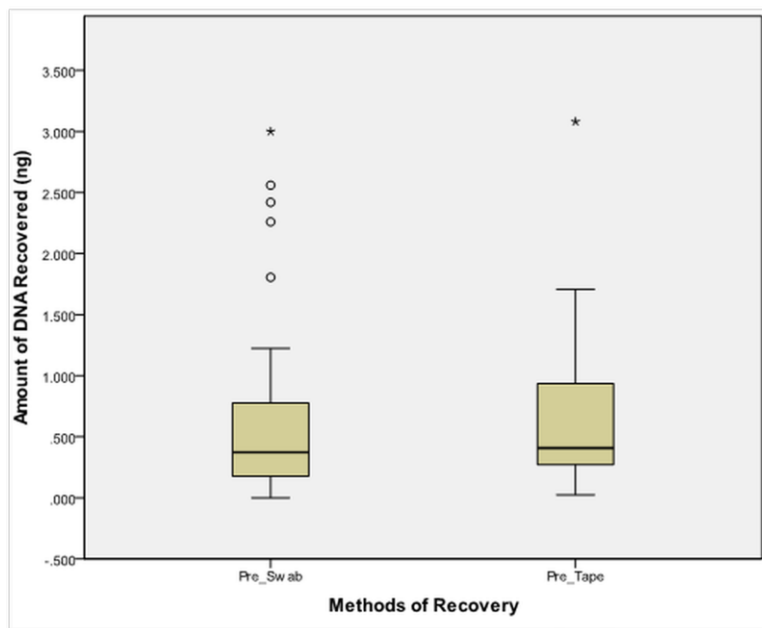


Fig. 1

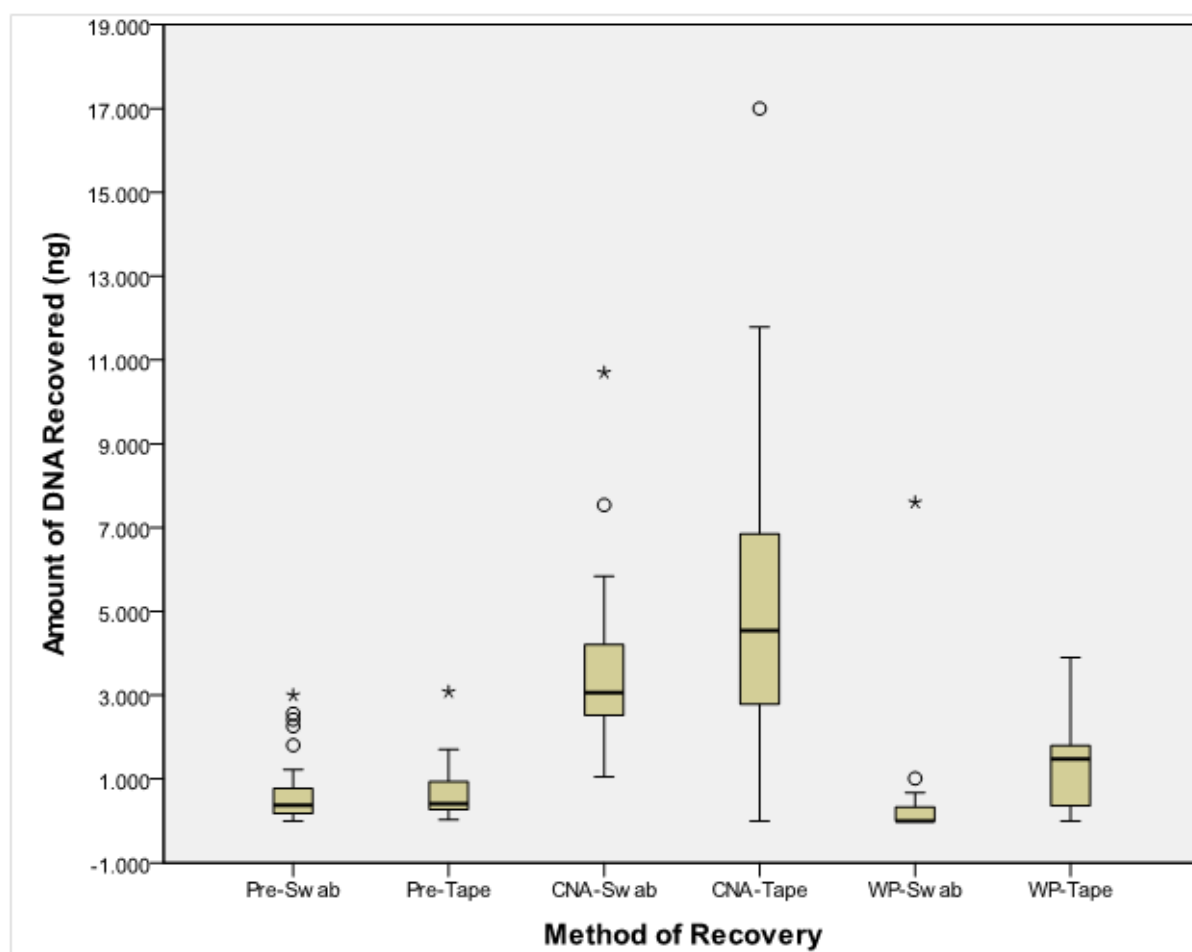


Fig. 2

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Novelty Statement**Touch DNA – the prospect of DNA profiles from cables**

Cable theft and metal theft cause widespread damage and disruption to the railway. At the present, no attempts on DNA analysis are made on the cables recovered, with investigators relying mainly on enhancement of latent fingerprints. To the best of our knowledge, no studies have been performed to investigate the deposition, recovery and profiling of touch DNA from such surfaces.

This manuscript demonstrates DNA typing of Touch DNA on rail cables after application of common finger mark enhancement reagents (cyanoacrylate fuming and wet powder suspensions), illustrating the importance in each step of 'localisation, recovery and typing' of touch DNA. The results reported suggest that DNA and fingerprint identification can be applied as a two-pronged approach in criminal investigations involving cable theft.

Highlights

Touch DNA – the prospect of DNA profiles from cables

- Recovery and profiling of touch DNA from the surface of cable sheaths was successful
- No significant difference between swabs or mini-tapes was seen
- CNA fuming performed better than WPS in terms of subsequent recovery and profiling of DNA